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(54) Title: ALLOGENEIC PARACRINE CYTOKINE TUMOR VACCINES

(57) Abstract

The present invention provides a method of treating cancer comprising (a) obtaining a tumor cell line, (b) modifying the tumor cell line to render it capable of producing an increased level of a cytokine relative to the unmodified tumor cell line, and (c) administering the tumor cell line to a mammalian host having at least one tumor that is the same type of tumor as that from which the tumor cell line was obtained, wherein the tumor cell line is allogeneic and is not MHC-matched to the host. The present invention also provides a pancreatic tumor cell line, a method and medium for obtaining such a tumor cell line, and a composition comprised of cells of a purified pancreatic tumor cell line.

ALLOGENEIC PARACRINE CYTOKINE TUMOR VACCINES

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

This invention was made with Government support under Grant Number CA62924 awarded by the National Institutes of Health, and under Grant Number CA57842 awarded jointly by the National Institutes of Health and National Cancer Institute. The Government may have certain rights in this invention.

TECHNICAL FIELD OF THE INVENTION

The present invention pertains to a method of treating cancer using allogeneic tumor cell lines, i.e., tumor cell lines that are genetically dissimilar to those of the host. In particular, the invention pertains to a method of treating pancreatic cancer using an allogeneic pancreatic tumor cell line. The present invention also pertains to a pancreatic tumor cell line, a method and medium for obtaining such a cell line, and a composition comprised of cells of a purified pancreatic tumor cell line.

BACKGROUND OF THE INVENTION

It is generally accepted that human tumor cells contain multiple specific alterations in the cellular genome responsible for their malignant phenotype. These alterations affect the expression or function of genes that control cell growth and differentiation. For instance, typically these mutations are observed in oncogenes, or positive effectors of cellular transformation, such as *ras*, and in tumor suppressor genes (or recessive oncogenes) encoding negative growth regulators, the loss of function of which results in expression of a transformed phenotype, such as *p53*, *Rb1*, *DCC*, *MCC*, *NF1*, and *WT1*.

Mutations have been detected in all of the common human tumors including pancreatic and colorectal

antigens. In a functioning immune system, tumor antigens are processed and expressed on the cell surface in the context of major histocompatibility complex (MHC) class I and II molecules, which, in humans, also are termed "human-leukocyte associated" (HLA) molecules. When complexed to antigens, the MHC class I and II molecules are recognized by $CD8^+$ and $CD4^+$ T cells, respectively. This recognition generates a set of secondary cellular signals and the paracrine release of specific cytokines, or soluble so-called "biological response modifiers", that mediate interactions between cells and stimulate host defenses to fight off disease. The release of cytokines then results in the proliferation of antigen-specific T cells.

Thus, active immunotherapy involves the injection of tumor cells, typically in the vicinity of a tumor, to generate either a novel or an enhanced systemic immune response. The ability of this immunotherapeutic approach to augment a systemic T cell response against a tumor has been previously disclosed, e.g., amongst others, see International Application WO 92/05262, Fearon et al., Cell, 60, 397-403 (1990), and Dranoff et al., Proc. Natl. Acad. Sci., 90, 3539-3543 (1993). The injected tumor cells are usually altered to enhance their immunogenicity, such as by admixture with non-specific adjuvants, or by genetic modification of the cells to express cytokines, or other immune co-stimulatory molecules. The tumor cells employed can be autologous, i.e., derived from the same host as is being treated. Alternately, the tumor cells can be MHC-matched, or derived from another host having the same, or at least some of the same, MHC complex molecules.

Clinical researchers prefer the use of autologous over MHC-matched tumor cells, and *vice versa*, for different reasons. Namely, autologous cells are preferred since each patient's tumor expresses a unique set of tumor antigens that can differ from those found on histologically-similar, MHC-matched tumor cells from another patient, see, e.g., Kawakami et al., J. Immunol., 148, 638-643 (1992); Darrow

tumor antigens on their MHC class I and II molecules, and directly activate the T cell arm of the immune response. In contrast, the results of Huang et al. indicate that the professional APCs of the host rather than the vaccinating tumor cells prime the T cell arm of the immune response. The tumor vaccine cells secrete a cytokine such as GM-CSF and recruit to the region of the tumor bone marrow-derived APCs. The bone marrow-derived APCs take up the whole cellular protein of the tumor for processing, and then present the antigenic peptide(s) on their MHC class I and II molecules. In this fashion, the APCs prime both the $CD4^+$ and the $CD8^+$ T cell arms of the immune system, resulting in the generation of a systemic antitumor immune response that is specific for the antigenic epitopes of the host tumor. These results suggest that it may not be necessary to use autologous or MHC-matched tumor cells in cancer treatment.

Other results suggest that the transfer of allogeneic MHC genes (i.e., genes from a genetically dissimilar individual of the same species) can enhance tumor immunogenicity. Specifically, in certain cases, the rejection of tumors expressing allogeneic MHC class I molecules resulted in enhanced systemic immune responses against subsequent challenge with the unmodified parental tumor, as reviewed in Jaffee et al., *supra*, and Huang et al., *supra*. This appears to represent an example of the general phenomenon described as "xenogenization" by Itaya et al., Cancer Res., 47, 3136-3140 (1987), wherein tumor vaccine potency is enhanced by introducing genes into the tumor cell that code for foreign antigens.

Thus, there remains a need for a method of treating cancer, in particular, a method of treating pancreatic cancer, which does not rely on use of autologous or MHC-matched tumor cells, and that avoids the difficulties and shortcomings associated with such use. The present invention provides such a method, as well as components necessary for effectuating the method. These and other objects and advantages of the present invention, as well as

response. In particular, a therapeutic response is a systemic immune response (i.e., a T cell response) to tumor antigens. Such a response can be assessed by monitoring the attenuation of tumor growth and/or tumor regression. "Tumor growth" includes an increase in tumor size and/or the number of tumors. "Tumor regression" includes a reduction in tumor mass.

"Cancer" according to the invention includes cancers, in particular those of epithelial origin, characterized by abnormal cellular proliferation and the absence of contact inhibition, which can be evidenced by tumor formation. The term encompasses cancer localized in tumors, as well as cancer not localized in tumors, such as, for instance, cancer cells which expand from a tumor locally by invasion. Thus, any type of cancer can be targeted for treatment according to the invention. For example, the approach preferably can be applied in several clinical scenarios including, but not limited to, local adjuvant therapy for resected cancers, and local control of tumor growth, such as carcinomas of the bladder, breast, colon, kidney, liver, lung, ovary, pancreas, rectum, and stomach. The method also preferably can be used for treatment when the tumor is a sarcoma (e.g., a fibrosarcoma or rhabdosarcoma), a hematopoietic tumor of lymphoid or myeloid lineage, or another tumor, including, but not limited to, a melanoma, teratocarcinoma, neuroblastoma, or glioma.

Preferably the method of the invention can be employed to treat pancreatic cancer. Thus, the present invention also provides a method of treating pancreatic cancer comprising the steps of (a) obtaining a pancreatic tumor cell line, (b) modifying the tumor cell line to render it capable of producing an increased level of a cytokine relative to the unmodified tumor cell line, and (c) administering the tumor cell line to a mammalian host having at least one pancreatic tumor, wherein the tumor cell line is allogeneic and is not MHC-matched to the host.

sterile container and moved to a laboratory laminar flow hood. The portion of the tumor to be employed for isolation of tumor cell lines can be minced into small pieces; the remainder of the tumor can be stored at -70°C. The slices of tumor then can be digested into single cell suspensions using a solution of Collagenase I. This digestion can be carried out at room or at elevated temperature. Preferably the digestion is carried out at 37°C, while shaking the mixture, e.g., in a shaking incubator.

The single cell suspension is then pelleted, and the pellets can be resuspended in a small volume of tissue culture medium. The resuspended cells can be inoculated into tissue culture medium appropriate for the growth of the cells in culture at a density of about 2 x 10^5 tumor cells/ml. Preferably the medium is one that has wide applicability for supporting growth of many types of cell cultures, such as a medium that utilizes a bicarbonate buffering system and various amino acids and vitamins. Optimally the medium is RPMI-1640 medium. The medium can contain various additional factors as necessary, e.g., when required for the growth of tumor cells, or for maintenance of the tumor cells in an undifferentiated state.

The cultures can be maintained at about 35-40°C in the presence of about 5-7% CO_2. The tumor cell cultures can be fed and recultured as necessary, i.e., typically every 1 to 10 days. The tumor cells also can be subjected to differential trypsinization to remove other cells (e.g. stromal cells) that can overgrow the primary tumor cultures. Preferably, such differential trypsinization is done about every 5 to 10 days.

When it appears that a substantially pure culture of the tumor cells has been obtained, various tests can be carried out as necessary to determine the relative purity of the cultures, and to characterize the resultant tumor cell lines. For instance, the existence of certain genetic

(Indianapolis, IN), Biofluids, Inc. (Rockville, MD), and other suppliers manufacturing similar products.

Preferably the tumor cell line (which desirably is a pancreatic tumor cell line) comprises a mutation in an oncogene or tumor suppressor gene such that the oncogenic nature of the tumor cell line, and its derivation from a host tumor, can be confirmed. The mutation can occur in any oncogene or tumor suppressor gene, including, but not limited to, *trk*, *ks3*, *hst*, *ras*, *myc*, *p53*, *mas*, *Rb1*, *DCC*, *MCC*, *NF1*, and *WT1*. Optimally the tumor cell line comprises a *ras* mutation. Preferably the mutation is present in codon 12, 13, or 61 of one of the *ras* genes H-*ras*, K-*ras*, and N-*ras*. Optimally the mutation is in codon 12 of a *ras* gene, preferably codon 12 of a K-*ras* gene.

The use of a tumor cell line characterized by a *ras* mutation is advantageous inasmuch as the mutations which render a *ras* gene oncogenic have been characterized, e.g., as reviewed by Bos, *supra*, and Barbacid, *supra*. This means that peptides that incorporate amino acid changes known to result in a *ras* oncoprotein can be synthesized easily, and can be evaluated as targets of cytotoxic T lymphocytes (CTLs). Host immune responses to these peptides can be assessed both before and after vaccination.

The tumor cell line, which preferably is a pancreatic cell line, also can be characterized by a further trait which distinguishes the tumor cells from other cells and can be employed, for instance, for monitoring cell survival either *in vitro* or *in vivo*. Examples of such a trait include antibody staining for a particular protein, which, desirably is a cell surface protein. Preferably the pancreatic tumor cell lines of the present invention demonstrate cytokeratin staining upon histochemical staining using an antibody directed against cytokeratin. Accordingly, the present invention provides preferred pancreatic tumor cell lines including, but not limited to Panc 4.14.93, Panc 1.28.94, Panc 6.3.94, Panc 8.13.94, Panc

those of ordinary skill in the art. Apprcpriate viral vectors include, but are not limited to simian virus 40, bovine papilloma virus, Epstein-Barr virus, adenovirus, herpes virus, vaccinia virus, Moloney murine leukemia virus, Harvey murine sarcoma virus, murine mammary tumor virus, and Rous sarcoma virus.

Reference to a vector or other DNA sequences as "recombinant" merely acknowledges the linkage of DNA sequences which are not typically conjoined as isolated from nature. A "gene" is any nucleic acid sequence coding for a protein or a nascent mRNA molecule. Whereas a gene comprises coding sequences plus any non-coding (e.g., regulatory sequences), a "coding sequence" does not include any non-coding DNA. A "promoter" is a DNA sequence that directs the binding of RNA polymerase and thereby promotes RNA synthesis. "Enhancers" are *cis*-acting elements of DNA that stimulate or inhibit transcription of adjacent genes. An enhancer that inhibits transcription also is termed a "silencer". Enhancers differ from DNA-binding sites for sequence-specific DNA binding proteins found only in the promoter (which also are termed "promoter elements") in that enhancers can function in either orientation, and over distances of up to several kilobase pairs (kb), even from a position downstream of a transcribed region.

Any suitable vector can be employed that is appropriate for introduction of nucleic acids into eukaryotic tumor cells, or more particularly animal tumor cells, such as mammalian, e.g., human, tumor cells. Preferably the vector is compatible with the tumor cell, e.g., is capable of imparting expression of the cytokine gene or coding sequence, and is stably maintained or relatively stably maintained in the tumor cell. Desirably the vector comprises an origin of replication. Preferably the vector also comprises a so-called "marker" function by which the vector can be identified and selected (e.g., an antibiotic resistance gene). When a cytokine coding sequence is transferred (i.e., as opposed to a cytokine

linked to) its own promoter, another promoter, including a constitutive promoter, such as, for instance the adenoviral type 2 (Ad2) or type 5 (Ad5) major late promoter (MLP) and tripartite leader, the cytomegalovirus (CMV) immediate early promoter/enhancer, the Rous sarcoma virus long terminal repeat (RSV-LTR), and others, can be employed to command expression of the cytokine coding sequence.

Alternately, a tissue-specific promoter (i.e., a promoter that is preferentially activated in a given tissue and results in expression of a gene product in the tissue where activated) can be used in the vector. Such promoters include but are not limited to the elastase I gene control region which is active in pancreatic acinar cells as described by Swift et al., Cell, 38, 639-646 (1984) and MacDonald, Hepatology, 7, 425-515 (1987); the insulin gene control region which is active in pancreatic beta cells as described by Hanahan, Nature, 315, 115-122 (1985); the hepatocyte-specific promoter for albumin or α_1-antitrypsin described by Frain et al., Mol. Cell. Biol., 10, 991-999 (1990) and Ciliberto et al., Cell, 41, 531-540 (1985); and the albumin and alpha$_1$-antitrypsin gene control regions which are both active in liver as described by Pinkert et al., Genes and Devel., 1, 268-276 (1987) and Kelsey et al, Genes and Devel., 1, 161-171 (1987).

Similarly, a tumor-specific promoter, such as the carcinoembryonic antigen for colon carcinoma described by Schrewe et al., Mol. Cell Biol., 10, 2738-2748 (1990), can be used in the vector. Along the same lines, promoters that are selectively activated at different developmental stages (e.g., globin genes are differentially transcribed in embryos and adults) can be employed for gene therapy of certain types of cancer.

Another option is to use an inducible promoter, such as the IL-8 promoter, which is responsive to TNF, or the 6-16 promoter, which is responsive to interferons, or to use other similar promoters responsive to other cytokines or other factors present in a host or that can be administered

increased over that observed for the unmodified (i.e., parental) tumor cell line. Even more preferably, the modified cell line produces a level of cytokine that results in cytokine secretion greater than 35 ng/10^6 cells/24 hours.

Administering the Modified Tumor Cell Line

"Administering" modified cells of the tumor cell line to a mammalian host refers to the actual physical introduction of the modified (i.e., cytokine-producing) tumor cells into the host. Any and all methods of introducing the modified tumor cells into the host are contemplated according to the invention; the method is not dependent on any particular means of introduction and is not to be so construed. Means of introduction are well known to those skilled in the art, and also are exemplified herein.

Preferably the modified tumor cell line is administered to a host having at least one tumor (i.e., the host can have more than one tumor) that is of the same type as that from which the cell line was obtained. "Same type of tumor" encompasses tumors which are histologically similar, i.e., similar in terms of the structure and property of the tissue/organ being treated. While it is anticipated that the administered tumor cell line can have some antigens (e.g., tumor antigens or MHC antigens) in common with the host tumor, for the purpose of this invention, it is not necessary that the administered tumor cell and the host tumor have any MHC antigens in common. Similarly, even though tumor antigens can differ between the administered tumor cell line and the host tumor, it is preferred that there is enough commonality such that administration of the tumor cell line can effect a systemic (i.e., a T cell-mediated) response against the host tumor. Accordingly, the present invention encompasses the administration of a tumor cell line which is allogeneic (i.e., genetically dissimilar) to the host, and which is

stimulatory functions, or, for example, admixture with nonspecific adjuvants including but not limited to Freund's complete or incomplete adjuvant, emulsions comprised of bacterial and mycobacterial cell wall components, and the like.

Methods of Use

The allogeneic tumor cell lines, particularly the allogeneic pancreatic tumor cell lines, can be used to vaccinate patients with histologically similar tumors for the purpose of generating a systemic antitumor immune response against the patient's own tumor.

To facilitate administration, a modified allogeneic tumor cell line (i.e, a modified allogeneic pancreatic tumor cell line) can be made into a pharmaceutical composition or implant appropriate for administration *in vivo*, with appropriate carriers or diluents, which further can be pharmaceutically acceptable. The means of making such a composition or an implant have been described in the art, see, for instance, Remington's Pharmaceutical Sciences, 16th Ed., Mack, ed. (1980). Where appropriate, a tumor cell line can be formulated into a preparation in solid, semisolid, liquid or gaseous form, such as a tablet, capsule, powder, granule, ointment, solution, suppository, injection, inhalant, or aerosol, in the usual ways for their respective route of administration. Means known in the art can be utilized to prevent or minimize release and absorption of the composition until it reaches the target tissue or organ, or to ensure timed-release of the composition. Preferably, however, a pharmaceutically acceptable form is employed which does not ineffectuate the compositions of the present invention. Thus, desirably a modified allogeneic tumor cell line (i.e., a modified allogeneic pancreatic tumor cell line) can be made into a pharmaceutical composition comprising a balanced salt solution, preferably Hanks' balanced salt solution.

predetermined amount of the composition, alone or in appropriate combination with other active agents. The term "unit dosage form" as used herein refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of the compositions of the present invention, alone or in combination with other active agents, calculated in an amount sufficient to produce the desired effect, in association with a pharmaceutically acceptable diluent, carrier, or vehicle, where appropriate. The specifications for the novel unit dosage forms of the present invention depend on the particular pharmacodynamics associated with the pharmaceutical composition in the particular host.

Preferably a sufficient number of the modified tumor cells are present in the composition and introduced into the host such that expression of cytokine by the host cell, and subsequent recruitment of APCs to the tumor site results in a greater immune response to the extant host tumor than would otherwise result in the absence of such treatment, as further discussed herein. Accordingly, the amount of host cells administered should take into account the route of administration and should be such that a sufficient number of the tumor cells will be introduced so as to achieve the desired therapeutic (i.e. immunopotentiating) response. Furthermore, the amounts of each active agent included in the compositions described herein (e.g., the amount per each cell to be contacted or the amount per certain body weight) can vary in different applications. In general, the concentration of modified tumor cells preferably should be sufficient to provide at least from about 1×10^6 to about 1×10^9 tumor cells, even more preferably, from about 1×10^7 to about 5×10^8 tumor cells, although any suitable amount can be utilized either above, e.g., greater than 5×10^8 cells, or below, e.g., less than 1×10^7 cells.

Example 1

This example illustrates the method of obtaining and culturing the allogeneic tumor cell lines of the present invention.

Eleven allogeneic pancreatic tumor cell lines were developed from patients undergoing pancreaticoduodenectomy at Johns Hopkins Hospital. These cell lines were generated from fresh human pancreatic tumor explants obtained at the time of surgical resection. Namely, immediately upon tumor resection, the specimen was placed on ice in a sterile container and moved to a laminar flow tissue culture hood in a laboratory. All subsequent manipulations were performed using standard sterile tissue culture technique, and using media and reagents from various commercial suppliers (e.g. JRH Biosciences (Lenexa, KS), Gibco BRL (Gaithersberg, MD), Hyclone Labs. (Logan, UT), Sigma Biosciences (St. Louis, MO), Cell Sys. Corp. (Kirkland, WA), Intergen Co. (Purchase, NY), Eli Lilly and Co. (Indianapolis, IN), Biofluids, Inc. (Rockville, MD), and other suppliers manufacturing similar products).

The portion of the tumor to be employed for isolation of tumor cell lines was minced into small pieces measuring about a few millimeters in diameter. The pieces were placed in a solution containing about 15 mg of Collagenase I, and were digested at 37°C in a shaking incubator into single cell suspensions. The pancreatic tumor cell suspensions were then subjected to gravity centrifugation for five minutes to pellet the cells. The pellets were resuspended and plated by inoculating into a 25 cm^2 tissue culture flask with about 1-2 x 10^6 viable cells in RPMI 1640 medium containing 20% fetal bovine serum, 100 units (U) of human insulin per 500 ml of medium, and 5 μg per 500 ml of medium of each of the insulin-like growth factors 1 and 2. The cultures were placed in 25 cm^2 tissue culture flasks and were incubated at about 37°C in humidified incubators with about 5-7% CO_2.

(i.e., Panc 10.5.92 and Panc 9.6.94) also express elevated levels of MHC class II antigens. The pancreatic tumor cell lines are easily expanded in culture and have doubling times of about 72 hours.

The methods employed in this example for derivation of allogeneic pancreatic tumor cell lines similarly can be employed for the generation and isolation of other kinds of allogeneic tumor cell lines.

Example 2

This example illustrates the method of modifying the allogeneic tumor cell lines of the present invention to produce an increased amount of a cytokine. Inasmuch as the cytokine granulocyte-macrophage colony stimulating factor (GM-CSF) is potentially more potent than other cytokines in generating a systemic antitumor response in preclinical tumor models (Dranoff et al., Proc. Natl. Acad. Sci., 90, 3539-3542 (1993), the Panc cell line 10.5.92 described in Example 1 was employed as representative of the allogeneic tumor cell lines, and was modified to secrete GM-CSF.

To accomplish this, a recombinant human GM-CSF gene was cloned into pcDNA 1/Neo. All cloning reactions and DNA manipulations were carried out using methods well known to the ordinary skilled artisan, and which have been described in the art, e.g., Maniatis et al., Molecular Cloning: A Laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory, NY, (1982)). Enzymes employed in these reactions were obtained from commercial suppliers (e.g., New England Biolabs, Inc., Beverly, MA; Clontech, Palo Alto, CA; Boehringer Mannheim, Inc., Indianapolis, IN; etc.) and were used according to the manufacturers' recommendations.

The plasmid pcDNA 1/Neo contains the human GM-CSF cytokine coding sequence under the control of the cytomegalovirus (CMV) promoter, and the neomycin resistance gene also controlled by a separate CMV promoter. The CMV promoter was employed since it is able to drive a relatively high level of gene expression in most eukaryotic

Example 3

This example illustrates further studies regarding GM-CSF administration to a host.

Further studies confirm that GM-CSF secretion needs to parallel the known paracrine physiology of this cytokine. In particular, secretion must be at the site of the relevant antigens (i.e., the tumor cells), as described in the previous example, and high levels must be sustained for several days (Dranoff et al., *supra*; Golumbek et al., Cancer Research, 53, 1-4 (1993)). However, it appears that the tumor cell itself need not be the source of GM-CSF secretion (Golumbek et al., *supra*). Immunologic protection and histologic infiltrates similar to those seen with retrovirally transduced cytokine-expressing tumor cells can be generated when GM-CSF is slowly released from biodegradable polymers co-injected with the tumor cell. In addition, if a second non-cross reacting tumor is co-injected with a GM-CSF secreting tumor, immunologic protection against both tumors can be generated. Simple injection of soluble GM-CSF along with tumor cells, however, does not provide sustained local levels of this cytokine and does not generate systemic immunity (Golumbek et al., *supra*). Thus, the effectiveness of using an allogeneic tumor cell that was not MHC-matched to the host cell for delivery of cytokine *in vivo* was explored.

In murine models, it was demonstrated that the antitumor immunity generated with the delivery of GM-CSF by bystander allogeneic tumor cells is comparable to that achieved when GM-CSF is delivered by the target tumor cell itself. Specifically, in these experiments, BALB/c mice were subcutaneously vaccinated with irradiated CT26 colon carcinoma cells, with GM-CSF delivered either by retrovirally transduced CT26 cells, or by retrovirally transduced B16-F10 cells. Two weeks later, mice were rechallenged with injections of wild-type strain CT26. The CT26 tumor cell line possesses some intrinsic immunogenicity; however, a greater degree of protection was

cells *in vivo*. The modified tumor cells also can be altered to enhance their immunogenicity. For instance, the cells can be further genetically manipulated (e.g., through insertion of other cytokine or immune stimulatory nucleic acid sequences), or can be admixed with non-specific adjuvants (e.g., Freund's complete or incomplete adjuvant, emulsions comprised of bacterial and mycobacterial cell wall components, etc.).

The invention can be used in mammals, particularly humans, having various tumors, for instance, a carcinoma of the bladder, breast, colon, kidney, liver, lung, ovary, pancreas, rectum, or stomach; a hematopoietic tumor of lymphoid or myeloid lineage; a tumor of mesenchymal origin such as a fibrosarcoma or rhabdomyosarcoma; or another tumor, including a melanoma, teratocarcinoma, neuroblastoma, or glioma. Preferably, the invention can be used in the treatment of pancreatic cancer. It also is anticipated that the patient can be treated prior to, or in addition to (i.e., concurrently or immediately following) immunotherapy as described herein with any number of methods as are employed to treat cancer, for instance, surgical resection, irradiation, chemotherapy, and the like.

All of the references cited herein, including patents, patent applications, and publications, are hereby incorporated in their entireties by reference.

While this invention has been described with an emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations of the preferred embodiments can be used and that it is intended that the invention can be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the following claims.

7. The method of claim 1, wherein said tumor cell line comprises a mutation in an oncogene or tumor suppressor gene.

8. The method of claim 1, wherein said cytokine is GM-CSF.

9. A method of treating pancreatic cancer comprising:

(a) obtaining a pancreatic tumor cell line,

(b) modifying said tumor cell line to render it capable of producing an increased level of a cytokine relative to the unmodified tumor cell line, and

(c) administering said tumor cell line to a mammalian host having at least one pancreatic tumor, wherein said tumor cell line is allogeneic and is not MHC-matched to said host.

10. The method of claim 9, wherein said tumor cell line is administered in close proximity to said tumor.

11. The method of claim 9, wherein said tumor cell line is irradiated prior to administration.

12. The method of claim 9, wherein said tumor cell line is treated prior to administration to enhance its immunogenicity.

13. The method of claim 12, wherein said treatment comprises genetic manipulation or admixture with nonspecific adjuvants.

14. The method of claim 9, wherein said pancreatic cell line is obtained by:

(a) obtaining a sample of a pancreatic tumor from a mammalian host,

24. A method of obtaining the tumor cell line of claim 18, which comprises:

(a) obtaining a sample of a pancreatic tumor from a mammalian host,

(b) forming a single cell suspension from said tumor sample,

(c) pelleting said tumor cells, and

(d) plating said tumor cells.

25. The method of claim 24, wherein said tumor cells are plated in a growth medium comprising fetal serum, insulin, and insulin-like growth factors 1 and 2.

26. A growth medium for culturing pancreatic tumor cells comprising fetal serum, insulin, and insulin-like growth factors 1 and 2.

27. The medium of claim 26, wherein said medium comprises insulin at a concentration of from about 0.1 to about 1.0 U/ml, insulin-like growth factor 1 at a concentration of from about 0.005 to about 0.05 μg/ml, and insulin-like growth factor 2 at a concentration of from about 0.005 to about 0.05 μg/ml.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/20802

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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